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POINT-COUNTERPOINT

# Point-Counterpoint: Should We Be Performing Metagenomic Next-Generation Sequencing for Infectious Disease Diagnosis in the Clinical Laboratory?

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**INTRODUCTION** With established applications of next-generation sequencing in inherited diseases and oncology, clinical laboratories are evaluating the use of metagenomics for identification of infectious agents directly from patient samples, to aid in the diagnosis of infections. Metagenomic next-generation sequencing for infectious diseases promises an unbiased approach to detection of microbes that does not depend on growth in culture or the targeting of specific pathogens. However, the issues of contamination, interpretation of results, selection of databases used for analysis, and prediction of antimicrobial susceptibilities from sequencing data remain challenges. In this Point-Counterpoint, Steve Miller and Charles Chiu discuss the pros of using direct metagenomic sequencing, while Kyle Rodino and Melissa Miller argue for the use of caution.

**KEYWORDS** clinical laboratory, metagenomics, sequencing, whole genome

## POINT

The fundamental role of the clinical microbiology laboratory is to detect, to identify, and to characterize pathogens that cause infections in patients, to guide patient management. Over the years, the methods in use have changed dramatically and now include a variety of culture-based approaches, visualization by microscopy, antigen detection, serological methods, and molecular detection. Each of these methods has its strengths and weaknesses and detects a defined spectrum of organisms that are potentially able to be cultivated and/or detected. Improvements to these microbiological assays, such as enhanced culture media and multiplex PCR syndromic panels, have expanded the range of organisms detectable by the laboratory. Along the way, clinical microbiologists have had to adapt their techniques and understand the benefits and limitations of new technologies in order to provide the most accurate information to guide treatment decisions.

The advent of high-throughput sequencing methods, known as next-generation sequencing (NGS), allows the simultaneous characterization of millions of individual DNA fragments (or cDNA fragments from RNA), facilitating genome assembly and metagenomic analysis. The ability to broadly detect all classes of organisms directly from patient samples is the major advantage of metagenomic NGS (mNGS) methods over targeted methods such as multiplex PCR panels. However, significant concerns exist regarding the performance, validity, and clinical significance of the organisms detected using mNGS. These substantial challenges need to be addressed during the clinical development of mNGS assays for pathogen detection. In our experience, these challenges can be overcome if the laboratory is willing to commit the needed resources and has the appropriate expertise, making it feasible for clinical laboratories to perform

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mNGS for patient testing (1). Furthermore, the clinical benefits of mNGS patient testing in facilitating the diagnosis of neurological infections and informing treatment decisions have already been demonstrated in a prospective, multisite, diagnostic trial (2). In that study of 204 patients with undiagnosed meningitis, encephalitis, and/or myelitis, 13 (22%) of 58 infectious cases were diagnosed by mNGS testing alone, and actionable clinical management and treatment decisions were made on the basis of mNGS results in more than one-half of those cases. The broad applicability of mNGS methods in research laboratories across a variety of infectious syndromes and sample types has also been illustrated in a growing number of case reports and small case series (3). Laboratories have continued to develop and to validate mNGS assays to address a variety of infectious disease syndromes, such as sepsis and pneumonia, demonstrating high analytical sensitivity and detection of more organisms thought to be pathogenic than are detected by conventional methods (4, 5).

Reference and commercial laboratories are now offering mNGS assays in laboratories certified in accordance with the CLIA program, for patient diagnosis using selected sample types. There appears to be substantial demand from providers and patients for mNGS testing, likely based on the hope of uncovering previously undiagnosed infectious disease. In order to support the use of mNGS testing, analytical and clinical validation studies are needed. Validation of an unbiased mNGS assay is a challenging task, since all organism species and subtypes are potentially detectable. This also poses regulatory challenges, as it would be impractical to obtain and to test more than a handful of cases for each infectious organism for purposes of validation. Our solution has been to adopt a "representative organism" approach, using individual species to serve as models for detection of other pathogens within that category (e.g., DNA viruses, RNA viruses, Gram-negative bacteria, Gram-positive bacteria, yeasts, and molds) (1), with the expectation that similar organism types can be expected to behave similarly in the assay. A list of detected species, with the findings confirmed by alternative laboratory testing, can be maintained and expanded over time to establish the spectrum of organisms that can be reliably tested for and reported by the mNGS assay. A model for this approach may be the clinical validation of matrix-assisted laser desorption ionization–time of flight mass spectrometry-based assays for bacterial identification, for which reference databases of reportable organisms may be expanded with additional confirmed strains over time.

A significant potential limitation to the use of mNGS assays for infectious disease diagnostics is the possibility of false-positive results due to contamination. This issue is not restricted to mNGS testing; in fact, microbiology laboratories are very familiar with contamination, especially for culture-based and PCR assays, and have developed specific protocols to deal with contamination events. Sources of "contamination" in mNGS results include detection of normal human body flora or background contaminating organisms in laboratory reagents or the environment, as well as bioinformatic analysis or database errors, such as misannotations, that can lead to organism misidentification. A salient example is the discovery of xenotropic murine leukemia virus-related virus (XMRV) and its initial apparent association with chronic fatigue syndrome (6). Although XMRV was later shown to be a laboratory-derived recombinant virus not present in the human population and the association with chronic fatigue syndrome was disproven, there was emerging demand for clinical testing and even treatment decision-making on the basis of XMRV status. However, we have found that risk mitigation strategies can be effective in avoiding misinterpretation of mNGS results due to contamination; strategies include (i) addition of interpretive notes based on guidance from expert laboratory physicians, (ii) confirmation of organisms newly identified by mNGS using alternative methods, (iii) implementation of a contamination-monitoring strategy, such as a dedicated contaminant reference database, and (iv) institution of a real-time teleconference hosted by experienced laboratory directors and subspecialist physicians (clinical microbial sequencing board) to discuss results in clinical context with treating physicians (2). Ultimately, implementation of these strat-

egies is the responsibility of the clinical laboratory providing mNGS testing, to ensure that mNGS results are used appropriately to guide patient management and care.

With respect to the adoption of mNGS testing in clinical laboratories, the guidelines and standards for clinical NGS testing that were initially developed for oncology and inherited disease applications (7, 8) have been adapted to infectious disease diagnostic performance evaluation (9). Conceptually, mNGS assays for infectious diseases are similar to oncological and noninvasive prenatal testing NGS assays, which are currently FDA approved and in widespread use, in their ability to detect unlimited numbers of sequence variants, and sample-processing steps in generating and analyzing sequence data overlap significantly. Thus, we may be able to leverage the requirements for testing and reporting of results for clinical mNGS assays that have already been established by our colleagues in other fields of medicine.

Quality control (QC) for mNGS assays is critical to ensure accurate performance. Because these are complex multistep tests, several QC metrics are required, including sample controls, external controls, internal controls, library quality metrics, sequencing quality metrics, and contamination controls. Each of these can be defined by the laboratory with acceptable criteria to ensure that sample quality and run quality are adequate and errors have not occurred during the processing steps. Although complex, the establishment and monitoring of robust mNGS QC metrics have been successfully adapted to the clinical microbiology laboratory setting (1, 10).

Analysis of mNGS data relies on the ability of bioinformatic pipelines to accurately classify sequence reads using established databases. Several bioinformatic pipelines have been developed for metagenomic analysis (11). Methods to filter out database errors and inaccurate sequence matches to avoid inadvertent misclassification are often necessary, especially when noncurated databases are used. Such methods include computational host subtraction, masking or removal of known errors, taxonomic classification, and filtering of sequence reads to ensure accuracy using alternative alignment programs. Another challenge is that application and validation of bioinformatic tools for mNGS analysis generally require specialized computational expertise. However, these challenges have been successfully addressed in the field of metagenomics with the development of easy-to-use, automated software with graphical user interfaces, including platforms such as SURPI+, Taxonomer, CosmosID, and OneCodex. In addition, the recent availability of databases that have been extensively curated for accuracy, such as FDA-ARGOS (12), has been useful in ensuring that mNGS results are reliable and correct.

The interpretation of mNGS results in clinical context is a key part of the use of MNGS testing in patient management. An advantage of this unbiased approach is the ability to diagnose rare infections, such as hepatitis E virus-associated meningoencephalitis (13). From a public health perspective, mNGS testing can provide evidence of newly emerging or reemerging pathogens. For example, our clinical mNGS testing of cerebrospinal fluid resulted in identification of the first human case of St. Louis encephalitis virus in California since 1986 (14). Unusual organism detections such as this can be reported to public health agencies, which may be able to assist in follow-up confirmatory testing.

Studies regarding the clinical utility and cost-effectiveness of mNGS assays for pathogen detection are lacking, making this area one in need of further investigation. While mNGS testing is expensive, relative to most traditional microbiological tests, the incremental costs of testing are minimal, compared to the costs of intensive care unit stays or invasive diagnostic biopsy procedures or even relative to the myriad diagnostic tests that are ordered for complex cases. Thus, if even a small percentage of mNGS test results demonstrate a causative pathogen, with effects on subsequent patient care, substantial cost savings for health systems overall are likely. Since mNGS testing for infectious disease diagnosis lacks a specific CPT code, reimbursement for direct testing costs is limited; therefore, the cost impact is best seen in the context of all patient management costs until further cost-benefit data are available to guide reimbursement decisions.

The sequencing data provided by mNGS may be able to provide clinically useful information beyond mere identification of the presence or absence of a potential pathogen. mNGS data have been leveraged to classify infections on the basis of host response, to characterize antimicrobial resistance, and to genotype detected pathogens for infection control and public health purposes. While negative results for typical diagnostic tests can be used to exclude the possibility of infection only for the agents targeted, negative pan-pathogen mNGS results may indicate a lower likelihood of an infectious etiology, hence being potentially useful as "rule-out" results (although indirect tests such as serological assays may also be needed). The absence of detectable pathogen nucleic acids can potentially reassure the treating physician that a severe infection is unlikely to be present. Finally, new technologies such as nanopore sequencing may enable mNGS to be performed with a turnaround time of under 6 h, greatly increasing the window of clinically actionable results afforded by mNGS testing.

mNGS assays for infectious disease diagnosis have been developed in the CLIA environment by specialized laboratories using approaches and guidelines similar to those used for oncological and inherited disease NGS tests. Both published and pending reports indicate that mNGS testing increases the diagnostic yield for infectious diseases in patients, especially those with critical illness and/or immunocompromised status, and may be useful for early identification of emerging pathogens relevant to public health. Clinical mNGS assays that have been validated in CLIA-certified laboratories are now available for a number of infectious syndromes, including meningoencephalitis, sepsis, and pneumonia. Testing of patients who are utilizing high levels of health care resources is likely to be particularly effective, as timely identification of atypical and/or rare infections is possible with mNGS testing. The utility of mNGS may also extend to other applications in the future, including discrimination of causes of infections based on transcriptome profiling (RNA sequencing) of the patient host response, identification of antibiotic resistance genes, and rapid mNGS diagnosis within hours on portable platforms such as the nanopore sequencer. The broad-spectrum, direct detection capability of mNGS testing may also facilitate its use as a rule-out test for excluding infection in patients with suspicion for noninfectious etiologies such as autoimmune disease. With ongoing improvements in technology and eventual regulatory approval, costs and barriers to mNGS implementation will continue to decrease, enabling expanded access to testing by treating physicians.

**Steve Miller and Charles Chiu**

## REFERENCES


1. Miller S, Naccache SN, Samayoa E, Messacar K, Arevalo S, Federman S, Stryke D, Pham E, Fung B, Bolosky WJ, Ingebrigtsen D, Lorizio W, Paff SM, Leake JA, Pesano R, DeBiasi R, Dominguez S, Chiu CY. 2019. Laboratory validation of a clinical metagenomic sequencing assay for pathogen detection in cerebrospinal fluid. Genome Res 29:831–842. https://doi.org/10.1101/gr.238170.118.
2. Wilson MR, Sample HA, Zorn KC, Arevalo S, Yu G, Neuhaus J, Federman S, Stryke D, Briggs B, Langelier C, Berger A, Douglas V, Josephson SA, Chow FC, Fulton BD, DeRisi JL, Gelfand JM, Naccache SN, Bender J, Dien Bard J, Murkey J, Carlson M, Vespa PM, Vijayan T, Allyn PR, Campeau S, Humphries RM, Klausner JD, Ganzon CD, Memar F, Ocampo NA, Zimmermann LL, Cohen SH, Polage CR, DeBiasi RL, Haller B, Dallas R, Maron G, Hayden R, Messacar K, Dominguez SR, Miller S, Chiu CY. 2019. Clinical metagenomic sequencing for diagnosis of meningitis and encephalitis. N Engl J Med 380:2327–2340. https://doi.org/10.1056/NEJMoa1803396.
3. Simner PJ, Miller S, Carrol KC. 2018. Understanding the promises and hurdles of metagenomic next-generation sequencing as a diagnostic tool for infectious diseases. Clin Infect Dis 66:778–788. https://doi.org/10.1093/cid/cix881.
4. Blauwkamp TA, Thair S, Rosen MJ, Blair L, Lindner MS, Vilfan ID, Kawli T, Christians FC, Venkatasubrahmanyam S, Wall GD, Cheung A, Rogers ZN, Meshulam-Simon G, Huijse L, Balakrishnan S, Quinn JV, Hollemon D, Hong DK, Vaughn ML, Kertesz M, Bercovici S, Wilber JC, Yang S. 2019. Analytical and clinical validation of a microbial cell-free DNA sequencing test for infectious disease. Nat Microbiol 4:663–674. https://doi.org/10.1038/s41564-018-0349-6.
5. Charalampous T, Kay GL, Richardson H, Aydin A, Baldan R, Jeanes C, Rae D, Grundy S, Turner DJ, Wain J, Leggett RM, Livermore DM, O'Grady J. 2019. Nanopore metagenomics enables rapid clinical diagnosis of bacterial lower respiratory infection. Nat Biotechnol 37:783–792. https://doi.org/10.1038/s41587-019-0156-5.
6. Irlbeck DM, Vernon SD, McCleary KK, Bateman L, Klimas NG, Lapp CW, Peterson DL, Brown JR, Remlinger KS, Wilfret DA, Gerondelis P. 2014. No association found between the detection of either xenotropic murine leukemia virus-related virus or polytropic murine leukemia virus and chronic fatigue syndrome in a blinded, multi-site, prospective study by the establishment and use of the SolveCFS BioBank. BMC Res Notes 7:461. https://doi.org/10.1186/1756-0500-7-461.
7. Aziz N, Zhao Q, Bry L, Driscoll DK, Funke B, Gibson JS, Grody WW, Hegde MR, Hoeltge GA, Leonard DGB, Merker JD, Nagarajan R, Palicki LA, Robetorye RS, Schrijver I, Weck KE, Voelkerding KV. 2015. College of American Pathologists' laboratory standards for next-generation sequencing clinical tests. Arch Pathol Lab Med 139:481–493. https://doi.org/10.5858/arpa.2014-0250-CP.
8. Roy S, Coldren C, Karunamurthy A, Kip NS, Klee EW, Lincoln SE, Leon A, Pullambhatla M, Temple-Smolkin RL, Voelkerding KV, Wang C, Carter AB. 2018. Standards and guidelines for validating next-generation sequencing bioinformatics pipelines: a joint recommendation of the Association

for Molecular Pathology and the College of American Pathologists. J Mol Diagn 20:4–27. https://doi.org/10.1016/j.jmoldx.2017.11.003.
9. Food and Drug Administration. 2016. FDA draft guidance: infectious disease next generation sequencing based diagnostic devices: microbial identification and detection of antimicrobial resistance and virulence markers: draft guidance for industry and Food and Drug Administration Staff. Food and Drug Administration, Rockville, MD.
10. Schlaberg R, Chiu CY, Miller S, Procop GW, Weinstock G. 2017. Validation of metagenomic next-generation sequencing tests for universal pathogen detection. Arch Pathol Lab Med 141:776–786. https://doi.org/10.5858/arpa.2016-0539-RA.
11. Mulcahy-O'Grady H, Workentine ML. 2016. The challenges and potential of metagenomics in the clinic. Front Immunol 7:29. https://doi.org/10.3389/fimmu.2016.00029.
12. Sichtig H, Minogue T, Yan Y, Stefan C, Hall A, Tallon L, Sadzewicz L, Nadendla S, Klimke W, Hatcher E, Shumway M, Aldea DL, Allen J, Koehler J, Slezak T, Lovell S, Schoepp R, Scherf U. 2019. FDA-ARGOS is a database with public quality-controlled reference genomes for diagnostic use and regulatory science. Nat Commun 10:3313. https://doi.org/10.1038/s41467-019-11306-6.
13. Murkey JA, Chew KW, Carlson M, Shannon CL, Sirohi D, Sample HA, Wilson MR, Vespa P, Humphries RM, Miller S, Klausner JD, Chiu CY. 2017. Hepatitis E virus-associated meningoencephalitis in a lung transplant recipient diagnosed by clinical metagenomic sequencing. Open Fourm Infect Dis 4:ofx121. https://doi.org/10.1093/ofid/ofx121.
14. Chiu CY, Coffey LL, Murkey J, Symmes K, Samples HA, Wilson MR, Naccache SN, Arevalo S, Somasekar S, Federman S, Stryke D, Vespa P, Schiller G, Messenger S, Humphries R, Miller S, Klausner JD. 2017. Diagnosis of fatal human case of St. Louis encephalitis virus infection by metagenomic sequencing, California, 2016. Emerg Infect Dis 23:1694–1698. https://doi.org/10.3201/eid2310.161986.

## COUNTERPOINT

**With great power comes great responsibility.**
—Uncle Ben in *Spider-Man*

The power of untargeted metagenomic next-generation sequencing (mNGS) is undeniable. As molecular technologies continue to evolve, it is tempting to adopt them quickly in the diagnostic laboratory. In the case of mNGS, there are a number of analytical and practical challenges that must be addressed before mNGS becomes a widely adopted routine diagnostic test in the clinical microbiology laboratory. We think that current limitations in test performance, a lack of standardized methods for mNGS, and limited guidance on clinical utility make this method less useful and less accessible than it needs to be for use in routine clinical microbiology laboratories.

The general perception, particularly among medical providers, is that mNGS is exquisitely sensitive, such that it will yield a result when all other diagnostic test results are negative. While mNGS is more analytically sensitive than smears or cultures, in most cases it is not as sensitive as targeted nucleic acid amplification methods, in part due to the vast amount of human DNA/RNA that is sequenced and removed during computational analysis. Analytical sensitivity may also vary by pathogen (1). Additional factors affecting mNGS sensitivity include the timing of specimen collection, specimen stability, and source/cellularity, as well as methodological variables such as the extraction method, library preparation, and data analysis (1, 2). The current sensitivity of mNGS prohibits the technology from becoming a frontline diagnostic test. Methods that enable host nucleic acid depletion should offer improved sensitivity for the agnostic detection of infectious disease etiologies (3).

The specificity of mNGS testing can be affected during every testing phase (preanalytical, analytical, and postanalytical), because specimens can become contaminated with nucleic acids from microorganisms that are not the cause of the patients' infections. Specimens are collected in a wide range of collection devices and locations, which introduces variables that might affect specificity, including microbial DNA found in sterile collection devices and exogenous DNA introduced by the environment or personnel during specimen collection. During the technical performance of mNGS, negative controls and genome coverage controls can help identify exogenous DNA from reagents or laboratory processes. With some Illumina platforms, sequence reads can be assigned to the wrong index (i.e., index hopping) during the demultiplexing process, which can lead to false-positive results if not controlled for and filtered out during analysis (4). Additional specificity challenges arise during data analysis and sequence alignment, including misclassification, database errors, and identification of DNA not derived from the patient sample.

While analytical specificity can be addressed during verification and through implementation of appropriate controls, a greater concern with mNGS testing is its clinical specificity. Is the detected sequence specific to the patient's current disease process? Do we understand the half-life of pathogen nucleic acids or the impact of latency, and does this differ among hosts? How many reads (i.e., what level of positivity) are required

to report a positive finding, and is this different for various disease processes or hosts? It is important that results of mNGS testing be scrutinized with an understanding of the potential for false-positive results.

There are not yet FDA-cleared or approved tests using mNGS for infectious diseases. Therefore, each clinical laboratory must develop and validate a laboratory-developed test (LDT) that includes extraction, library preparation, sequencing, and analysis pipeline. Optimization and verification of the performance of the system, across diverse potential pathogens, present a new level of complexity, compared to targeted molecular techniques. Any change to a component of the LDT must be reverified, which makes the use of open source pipelines particularly challenging, since it is critical for databases to remain up to date. For routine implementation, mNGS testing will need to be in a closed system that has undergone regulatory review.

During validation, thresholds must be set for the minimum number and quality of reads for analysis, the ratio of organism reads from the sample to reads present in the negative control, and the number of reads required for a reportable result. Once thresholds are established, clinical validation will likely involve testing of known positive and/or negative patient samples. The majority of validation studies use residual, frozen specimens and not prospectively collected, fresh samples, which limits the assessment of specimen stability and temporal variability. Unexpected results will almost certainly be encountered and should be adjudicated by an additional method, an endeavor that can be problematic from both cost and feasibility standpoints (1).

Result interpretation should be carefully considered during clinical validation. Unlike traditional culture-based methods, for which decades of data and clinical correlations have led to established procedures for interpretation, the paucity of prospective analyses and clinical trials for mNGS testing leaves interpretation open. While the interpretation of results may be more straightforward for normally sterile sources, nonsterile sources pose additional challenges, such as the normal microbiome and asymptomatic carriage. Therefore, interpretation of mNGS results in the clinical context will likely require significant manual effort. Committee review of results (similar to a tumor board) seems like a responsible approach, but routine laboratories may find assembling such expertise a limiting factor.

A number of practical challenges also exist, limiting the broad implementation of mNGS. While advancements in technology have shortened the time needed to perform mNGS, the current turnaround time (TAT) (in-house, 2 days to 1 week; send out, 2 or more weeks) limits routine use of mNGS testing, because results may not be available within a clinically actionable time frame (2, 5). The achievable TAT is dependent on the expected volume of samples. While mNGS is inherently high throughput, the necessary number of reads per run may limit the number of samples that can be pooled. If fewer samples are multiplexed, then the TAT and cost per test are increased. This alone may limit the feasibility of in-house mNGS.

For any clinical laboratory running molecular assays, contamination is always a risk and a fear. We attempt to mitigate this risk with quality control, unidirectional workflow, and stringent decontamination protocols. With the unbiased detection of mNGS, the concern regarding contamination only increases. Exogenous DNA from any source, not just that specific to the assay's target, will appear in the sequencing data. It is likely that laboratories will need dedicated space designed specifically for mNGS, allowing minimal chance for contamination. Space limitations faced by many clinical laboratories make establishing a dedicated mNGS area a challenge.

Data storage and security represent another hurdle to routine use of mNGS. Data storage on the terabyte level has not previously been needed in clinical microbiology. More than simply storing the data, security must be ensured, because mNGS data contain HIPAA-protected patient information (6). This is complicated when open source or commercially available cloud-based pipelines are used, requiring the sharing of data with entities that are not subject to the same level of regulation as the clinical laboratory. The cost of storing and securing the data generated, while complying with the requirements of accrediting agencies, should not be understated.

Finally, the future of reimbursement for diagnostic use of mNGS remains unclear. In early 2018, CMS approved reimbursement for the use of NGS in oncology for their patients. A number of caveats accompanied this decision, one being that only FDA-approved/cleared testing was reimbursable, excluding LDTs. This decision, along with recent reimbursement challenges for syndrome-based molecular panels, makes for an uncertain future.

The power of mNGS is indisputable, providing insight into the microbial composition of a sample not previously achievable in clinical microbiology. We must work responsibly to harness its diagnostic power for the benefit of our patients while remaining good stewards of resources, regardless of whether mNGS is performed in-house or at a reference laboratory. Quite simply, for broad implementation of mNGS testing, industry partners need to develop *in vitro* diagnostic products for infectious disease metagenomics. The majority of laboratories do not have the bandwidth for mNGS test development, bioinformatics expertise, or the financial investment needed to validate a mNGS LDT (~$100,000 in supplies for cerebrospinal fluid mNGS testing) (2).

Currently, the role of mNGS is derived largely from case-level reports of retrospective diagnosis and proof-of-principle applications. We need method comparison studies to optimize LDTs and to normalize protocols between institutions (2, 7, 8). The development of predictive models is needed to improve automated and integrated data analysis, providing actionable reports for clinicians. Finally, and most importantly, we need to establish the clinical utility of mNGS testing (9). Studies like that by Wilson et al. demonstrate the ability of mNGS to detect a potential pathogen not otherwise found by conventional methods, providing a diagnosis for an additional 3 to 6% of patients (10). However, given the current cost of such assays, broad use coupled with low expected return confers a significant financial burden per actionable result. Additional well-designed outcome studies will help identify patient populations, clinical syndromes, and specific laboratory test parameters for which mNGS is most likely to provide additional diagnostic information. Such guidance will allow targeted use of mNGS testing, increasing the expected utility and ensuring that the power of the technology is used in the settings most likely to improve patient care (11).

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## REFERENCES


1. Schlaberg R, Chiu CY, Miller S, Procop GW, Weinstock G. 2017. Validation of metagenomic next-generation sequencing tests for universal pathogen detection. Arch Pathol Lab Med 141:776–786. https://doi.org/10.5858/arpa.2016-0539-RA.
2. Simner PJ, Miller S, Carroll KC. 2018. Understanding the promises and hurdles of metagenomic next-generation sequencing as a diagnostic tool for infectious diseases. Clin Infect Dis 66:778–788. https://doi.org/10.1093/cid/cix881.
3. Gu W, Miller S, Chiu CY. 2019. Clinical metagenomic next-generation sequencing for pathogen detection. Annu Rev Pathol 14:319–338. https://doi.org/10.1146/annurev-pathmechdis-012418-012751.
4. Illumina. 2017. Effects of index misassignment on multiplexing and downstream analysis. Illumina, San Diego, CA. https://www.illumina.com/content/dam/illumina-marketing/documents/products/whitepapers/index-hopping-white-paper-770-2017-004.pdf.
5. Rhoads DD. 2018. Lowering the barriers to routine whole-genome sequencing of bacteria in the clinical microbiology laboratory. J Clin Microbiol 56:e00813-18. https://doi.org/10.1128/JCM.00813-18.
6. Erlich Y, Narayanan A. 2014. Routes for breaching and protecting genetic privacy. Nat Rev Genet 15:409–421. https://doi.org/10.1038/nrg3723.
7. Goldberg B, Sichtig H, Geyer C, Ledeboer N, Weinstock GM. 2015. Making the leap from research laboratory to clinic: challenges and opportunities for next-generation sequencing in infectious disease diagnostics. mBio 6:e01888. https://doi.org/10.1128/mBio.01888-15.
8. Simner PJ, Miller HB, Breitwieser FP, Pinilla-Monsalve G, Pardo CA, Salzberg SL, Sears CL, Thomas DL, Eberhart CG, Carroll KC. 2018. Development and optimization of metagenomic next-generation sequencing methods for cerebrospinal fluid diagnostics. J Clin Microbiol 56:e00472-18. https://doi.org/10.1128/JCM.00472-18.
9. Miller MB, Atrzadeh F, Burnham C-AD, Cavalieri S, Dunn J, Jones S, Mathews C, McNult P, Meduri J, Newhouse C, Newton D, Oberholzer M, Osiecki J, Pedersen D, Sweeney N, Whitfield N, Campos J. 2019. Clinical utility of advanced microbiology testing tools. J Clin Microbiol 57:e00495-19. https://doi.org/10.1128/JCM.00495-19.
10. Wilson MR, Sample HA, Zorn KC, Arevalo S, Yu G, Neuhaus J, Federman S, Stryke S, Briggs B, Langelier C, Berger A, Douglas V, Josephson SA, Chow FC, Fulton BD, DeRisi JL, Gelfand JM, Naccache SN, Bender J, Dien Bard J, Murkey J, Carlson M, Vespa PM, Vijayan T, Allyn PR, Campeau S, Humphries RM, Klausner JD, Ganzon CD, Memar F, Ocampo NA, Zimmerman LL, Cohen SH, Polage CR, DeBiasi RL, Haller B, Dallas R, Maron G, Hayden R, Messacar K, Dominguez SR, Miller S, Chiu CY. 2019. Clinical metagenomic sequencing for diagnosis of meningitis and encephalitis. N Engl J Med 380:2327–2340. https://doi.org/10.1056/NEJMoa1803396.
11. Saubolle MA, Weissfeld AS, Kraft CS. 2018. Designing studies acceptable for abstraction and inclusion in evidence-based laboratory practice guidelines. J Clin Microbiol 57:e00842-18. https://doi.org/10.1128/JCM.00842-18.

## SUMMARY

Points of agreement

1. Direct sequencing of clinical specimens offers the potential for unbiased, rapid detection of pathogens that may not be viable in culture or detected by traditional laboratory methods.
2. mNGS testing is not a replacement for conventional microbiological testing, such as culture and serology.
3. A comprehensive validation for every organism detectable by sequencing is not possible, requiring new strategies in which representative pathogens are included in the validation study as proxies for all other targets.
4. Offering mNGS assays requires a significant financial investment from the laboratory and will necessitate new strategies for laboratory reimbursement.
5. Contamination of mNGS assays is a significant challenge, requiring a well-designed control strategy that evaluates contamination in all aspects of testing.

Issues to be resolved

1. Determining how pathogenic organisms can be differentiated from commensals when using sequencing will require new approaches for laboratory consultation with clinicians.
2. Cost-effectiveness and guidelines on when to perform mNGS need to be developed.
3. Regulatory hurdles and lack of insurance reimbursement make implementation of mNGS testing difficult.

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